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MASTER'S THESIS

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June 2015

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2015

ABSTRACT

COMPARING IMMUNOHISTOLOGIC AND DEMOGRAPHIC VARIABLES OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

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DMD, ORAL AND MAXILLOFACIAL PATHOLOGY, 2015

INTRODUCTION: Due to differences in demographics, course of disease, morphology, and survival rates, human papilloma virus-associated squamous cell carcinoma (HPV-SCC) and non-HPV associated squamous cell carcinoma, designated head and neck squamous cell carcinoma (HNSCC), can be considered distinct clinico-pathologic entities. Over the past two decades, there has been an increase in incidence in HPV-SCC and it is expected to continue as the behaviors and habits associated with this disease continue to parallel a changing culture.

PURPOSE: This retrospective cohort study investigation examined molecular pathways, which are a series of molecular interactions that lead to certain cell function or states, associated with the progression of HPV-SCC and HNSCC.

METHOD: For this interim analysis, 22 cases, diagnosed as HNSCC, were identified from existing tissue specimens. Immunohistochemistry was utilized to quantify the expression of p53, a tumor suppressor gene, epidermal growth factor receptor (EGFR), a commonly over-expressed and mutated gene in HNSCC, p16, a surrogate marker for HPV, and pS6 and pAKT437, two markers that represent the mTOR (mechanistic target of rapamycin) pathway. The degree of immunohistochemical staining and correlation with demographic patient data was used to compare HPV-associated and non-HPV associated squamous cell carcinomas.

RESULTS: A 19:3 male (85%):female ratio and a 50% predilection for Caucasians were observed. An average age of 52.6 years, which is in line with the literature, was also noted. A majority of the cases,55% (12/22), presented as stage IV at diagnosis. Of these stage IV lesions, 89% were p16 (HPV) positive. p16 was reactive in 60% (13/22), p53 was reactive in 36% (8/22), pS6 was insignificant among both p16 +/- populations, and 14% (3/22) exhibited both p16 and p53 reactivity.

CONCLUSION: Efforts to collect specimens and data, along with the ongoing immunohistochemical staining, will continue until 120 cases have been reached. The entire cohort will then be evaluated in its entirety by comparing the staining results with corresponding demographic data. Therefore, based on the patient's specific genomic state, the treatments resulting from this design could target their tumors specifically and directly at the same time possibly eliminating unnecessary treatments, target prevention, decrease morbidity, and reduce treatment costs.

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LIST OF ABBREVIATIONS

HNSCC: Head and neck squamous cell carcinoma

HPV: Human papilloma virus

EGFR: Epidermal growth factor receptor

mTOR: Mammalian target of Rapamycin

pRb: retinoblastoma tumor-suppressor

CHAPTER I

Introduction

The sixth most common cancer in the world is head and neck squamous cell carcinoma (HNSCC) [1]. Anatomical sites in this category include the oral cavity, oropharynx and larynx. It has an estimated incidence of 633,000 cases with 335,000 associated deaths annually [1,2]. This disease has typically been seen in heavy tobacco and alcohol users who are usually older males; however, HNSCC is appearing in younger patients without this history. Modern sexual practices, an increase in the number of sexual partners, and the human papilloma virus (HPV) are now risk factors in addition to alcohol and smoking. Oral-genital and oral-anal practices are the route of transmission to the oropharynx and oral cavity.

HPV associated and HPV non-associated squamous cell carcinomas (HPV-SCC and HNSCC) are histologic subtypes further classifying HNSCC. Potentially, these tumors can be sub-classified based on particular signaling pathways that are apparently aberrant. The identification of these subtypes, by recognition of these pathways, will potentially improve target-oriented therapeutics resulting in more positive patient outcomes [3]. This study, by evaluation of mTOR and EGFR pathways in particular, will explore the characteristics in HPV and non-HPV associated HNSCC and the therapeutic means by which to target these pathways.

Chapter II

Head and Neck Squamous Cell Carcinoma

A squamous cell is a flat, polygonal cell located in the most superior portion of certain types of epithelium. Its cytoplasm is almost entirely filled with keratin providing a physical protective barrier to external environments. The malignant proliferation of this cell is the disease known as squamous cell carcinoma and is the cancer-type most capable of local spread and distant or metastatic spread. The primary locations for this disease process includes skin, oral cavity, esophagus, urinary tract, prostate, lungs, vagina, and cervix. Of these sites, four make up the majority of the cases which include head and neck cancer, esophageal cancer, non-melanoma skin cancer, and non-small cell lung cancer [1]. HNSCC makes up 90% of all head and neck cancers which include, anatomically, cancers of the nasal and paranasal sinuses, nasopharynx, oral cavity, oropharynx, and larynx [1,2,3]. HNSCC can be further categorized according to its state of differentiation in degrees from well-differentiated to poorly differentiated. Its ability to produce keratin, corresponding to its degree of differentiation, is referred to as "keratinizing" or "non-keratinizing" squamous cell carcinoma.

Incidence and Etiology

HNSCC is the sixth most common cancer, globally, with an estimated 633,000 new cases, yearly, resulting in 335,000 annual deaths [1,2,3]. Geographically, North America, Southeast Asia, Central and Western Europe, and Australia have the highest rates of incidence [2]. These tumors have commonalities such as male predominance, occurrence at 5th to 6th

decade, tobacco-alcohol association, betel nut chewing, HPV infection and are similar histologically [3,4].

Alcohol and tobacco

The synergistic effect of alcohol and tobacco use is the most important risk factor for developing HNSCC, though the mechanism by which they interact is still unclear. Alcohol(or ethanol), is not a carcinogen per se; however, it acts as a co-carcinogen by generating oxygen free radicals during its metabolism [5]. This process damages epithelial membrane constituents, alters DNA tumor-suppressor pathways, and amplifies intercellular signaling cascades causing chronic inflammation [5]. The effect of smoking on disease incidence is influenced by: the type of tobacco used (i.e. black tobacco), the age of smoking onset (i.e. longer duration), the number of cigarettes smoked per day, and deep inhalation [3]. The thousands of chemicals in tobacco leaves, and in particular, their combustion products, confer mutagenic effects on the cells of the head and neck mucosa [5]. The risk of HNSCC from smokeless tobacco is low in men and essentially non-existent in women in the United States. Due to mode of use and tobacco type, it is of increased significance in areas of India and Southeast Asia [5]. The "chewing" of betel quid is the most common etiological factor in these parts of the world. Betel quid is a combination of betel leaf, areca nut, and slaked lime. Often, tobacco is added and the product is known as *gutka*. It is consumed by placing the mixture between the cheek and gum where it is sucked or chewed and the chemically-infused saliva is either expectorated or swallowed. The use of gutka or betel quid imparts a euphoric, stimulating, but relaxing sensation which plays a major role in the abuse and dependence of this combination of substances. Alkaloids from the areca nut stimulate fibroblasts to produce collagen leading to fibrosis. This process is

termed*oral submucous fibrosis* and can occur anywhere from the oral cavity to the superior portion of the esophagus. This fibrosis undergoes transformation to squamous cell carcinoma at a rate of 7.6% over a 17 year period [5].

Human papilloma virus (HPV)

Human papilloma virus is the most common sexually transmitted infection in the United States. There are over two hundred types of HPV; most are not harmful to humans. About forty types can infect the genital, oropharyngeal, and nasopharyngeal areas and of these, only nine are associated with cancer. The two types considered highest risk are HPV 16 and 18 which have a well-established association with cervical cancers. However, HPV 16 accounts for the majority of HPV-associated HNSCC [2,3,6,7]. It has been recently shown that HPV infection is seen in a significant portion of HNSCC cases occurring in the oropharynx. This carcinomas has a particular prevalence for tissues making up Waldeyer's ring specifically, the lingual, palatine, and pharyngeal (adenoid) tonsils. These cancers are now subdivided into HPV associated (HPV-SCC) and non-HPV associated squamous cell (SCC) carcinomas.

HPV-SCC is a distinct entity which varies from conventional HNSCC in its demographics, behavior associations, and prognosis. Compared to conventional type, HPV-SCC occurs at a younger age of onset (below 40), is associated with specific sexual behaviors, and demonstrates an improved survival rate [8]. In the last twenty-five years, there has been an increase in incidence of HPV-SCC with a relatively stable number of cases of conventional HNSCC. At this rate, the number of HPV-SCC's among males will surpass the incidence of cervical cancers by the year 2020 [3]. Some studies have demonstrated a positive and synergistic correlation between HPV, alcohol, and tobacco while others have failed to find any association

[3,11,12]. Tobacco, alcohol, and HPV are risk factors that function independently or in combinations with one another [5].

When compared to HNSCC, HPV-SCC follows a different disease course and, as a result, treatment due to its unique pathogenesis [2,3,10]. The protein products of two oncogenes, designated E6 and E7, located in the HPV genome, play a significant role in the carcinogenesis of HPV-SCC. Under circumstances not completely understood, the E6 oncoprotein binds to p53, which promotes its degradation. The p53 molecule is a tumor suppressor protein that manages the cell cycle preventing catastrophic, uncontrolled, neoplastic mitosis. This protein is aptly referred to as the "guardian of the genome". Without its surveillance, risk of tumor formation is greatly increased. In a similar fashion, the E7 oncoprotein binds to the pRb protein, another impactful suppressor protein promoting tumor degradation. With low levels of pRb protein, which is also a negative regulator of p16, another tumor suppressor protein, tumor cells can be evaluated indirectly for HPV infection. Hence, immunohistochemical identification of p16 is used as a surrogate marker for HPV [2,3,13,14,15].

HNSCC's pathogenesis varies from HPV-SCC in many ways though deactivating p53 mutations are detected in approximately 21% of cases [16]. This process begins from a single cell progenitor which undergoes uncontrolled, monoclonal mitotic divisions expressing genetic and phenotypic alterations leading to dysplasia and finally, invasive carcinoma. Alterations such as tumor suppressor inactivation and proto-oncogene activation, though similar to HPV-SCC, are accomplished by genetic deletion, point mutations, gene amplification, and loss of heterozygosity [LOH]. LOH, which is the loss of one of the two gene copies, may result in a mutated remaining copy, is the most common genetic alteration in HNSCC and is found in approximately 70-80% of cases [8]. This genetic anomaly occurs early in HNSCC development

and is seen in 30% of squamous hyperplasias. The lost chromosomal allele is located at 9q21 which codes for cyclin-dependent kinase inhibitor 2A (CDKN2A), a tumor suppressor gene which codes for the tumor suppressor proteins p16 and p14. These proteins manage the G1 cell cycle and, in HPSCC, p16 is inactivated as opposed to being over-expressed in HPV-SCC. Further along in its progression from dysplasia to invasive SCC, LOH at 17p13 and p53 point mutations occur in 50% of HNSCC [8]. Other anomalies, such as amplification of 11q13 and cyclin D1 are also observed in 40% of dysplastic and 30-60% of HNSCC which confers a poorer prognosis and increased risk of metastasis. The above progression of events allows an individual cell to go from G1 to S phases in the cell cycle allowing for uncontrolled and potentially catastrophic proliferation [17].

Pathways of Pathogenesis

Epidermal Growth Factor Receptor

The epidermal growth factor receptor (EGFR) is a protein on the cell surface that plays a vital role in cellular growth, metabolism, and proliferation. Growth factor proteins, such as EGF (epidermal growth factor), TGF-α (tissue growth factor-alpha), and HB-EGF (heparin-binding epidermal growth factor), activate the EGFR pathway to initiate a cascade of molecular reactions resulting in cell proliferation via DNA synthesis.

Compared to normal mucosa, over expression of EGFR is seen in 90% of HNSCC tumor cells and is associated with more aggressive tumors and decreased rates of survival. Therefore, targeting and inhibiting this pathway has been shown to increase survival especially with a therapeutic antibody, cetuximab, which binds to EGFR and competes with its indigenous ligands to prevent activation. However, resistance to EGFR inhibition usually occurs by means not

entirely understood, such as utilizing other pathways to avoid apoptosis and allow continued growth [18].

mTor

The mechanistic target of rapamycin (mTOR) is a metabolic cellular pathway with multiple functions such as apoptosis, protein synthesis, and also plays a crucial role in cell survival. By promoting growth and metabolic pathways, recent studies have shown that mTOR is significantly over-activated in HPV positive and negative HNSCC [8]. EGFR-dependent and independent pathways activate this pathway. MTOR is a two-protein complex, protein kinase. The two proteins, TORC1 and TORC2, are downstream phosphorylation targets of pS6 and pAKTS473, respectively.

Rapamycin and RAD001 are therapeutic mTOR inhibitors which have demonstrated tumor burden reduction in laboratory animals. Evaluation of mTOR inhibitors may offer a potential mechanism to prevent HNSCC.

Clinical and Histologic Features

HNSCC usually develops from a leukoplakic or erythroplakic lesion most frequently in the areas of the lateral and ventral tongue and floor of the mouth. Leukoplakia is a clinical expression describing a white plaque or patch that cannot be identified as any other singular entity. It is the most common precursor to HNSCC and has a conversion rate of 1-2% per year [4]. Erythroplakia are red patches or plaques that, likewise, cannot be categorized as any other disease process. These lesions are red, or mixed (erythroleukoplakias), and have a greater risk (90%) of transformation to dysplasia, carcinoma in situ, or SCC [19].

HPV-SCC is seen, clinically, in the region known as Waldeyer's ring. It is most often found in the tonsillar crypts of the palatine and lingual tonsils. This location is unfortunate because the patient is usually unaware of its presence [19]. Patients usually become aware of the lesion due to dysphagia (difficulty in swallowing) or pain. As a result, these lesions tend to be larger and are more likely to have metastasized, compared to HNSCC, when diagnosed [19].

Microscopically, leukoplakia, the precursor lesion to SCC, is usually altered cytologically and/or architecturally. Early on, the lesion is characterized by simple hyperplasia, a thickening of the epithelium due to increased numbers of cells in the basal and parabasal strata. There is no change in the cytomorphology of the cells. When these architectural changes are combined with cytologic change, the term dysplasia is used. The cytologic criteria of dysplasia include a loss of polarity, an increased nuclear-to-cytoplasm ratio, and an increase in abnormal mitotic activity above the basal and parabasal cell levels. Dysplasic change progresses from the deepest (basal) layer to the most superficial (keratin or stratum corneum) layer. Depending on the layer in which cytologic change occurs, dysplasia can be qualified as mild, moderate, and severe. Mild dysplasia is limited to the lower third. Changes extending to the middle third and greater than two thirds, are classified as moderate and severe, respectively. The expression *carcinoma in situ* is reserved for dysplasia that extends the entire thickness but does not invade the basement membrane. A diagnosis of SCC is only made when invasion of the basement membrane and the underlying connective tissue can be unmistakably identified. The lesion is then graded according to its extent of differentiation. Well differentiated SCC has features still recognizable from normal squamous mucosa such as intracellular bridging, keratin pearl formation, and minimally ordered stratification. These three features cannot be identified in poorly differentiated SCC. A

moderately differentiated SCC falls somewhere in between. The less differentiated the tumor, the more prone it is to metastasis and to carry a poor prognosis.

Contrasting HNSCC, HPV-SCC does not typically involve the epithelial surface and progressive dysplasias are not observed. Because hyperkeratinization usually does not occur, HPV-SCC is sometimes referred to as non-keratinizing squamous cell carcinoma. This lesion invades as lobules of basoloid tumor cells which are often infiltrated by lymphocytes. Using immunohistochemistry, cells reactive to the p16 tumor suppressor protein marker confirms the presence of HPV infection. In addition, *in situ* hybridization may be performed to confirm the presence of HPV 16/18 [8].

Treatment and Prognosis

A more favorable prognosis exists for HPV-SCC where the five year survival rate approaches 60%. In HNSCC, the survival rates drop closer to 46% [7]. Other studies have demonstrated similar trends. HPV-SCC was reported to have a 28% reduction in risk of death and a 49% reduction in risk of recurrence when compared to HNSCC [7]. It is thought that the poorest prognoses for HNSCC's may be in part due to molecule-genetic changes such as mutation in p53 and over expression of EGFR [8]. It is also thought that the more favorable prognosisfor HPV-SCC is to its harboring less p53 mutations [8]. There is also speculation that the immune response to the HPV oncoproteins E6/E7 may contribute to superior survival rates for HPV-SCC even though this mechanism still remains unclear [2,21].

Even though HNSCC and HPV-SCC are genetically and epidemiologically distinct, most clinical trials have not differentiated them as such when it comes to treatment [2,13]. Both subtypes are treated with combinations of surgery, chemotherapy, and/or radiation therapy. In

order to design such an entity-specific protocol, molecular targeting strategies are required to focus treatment to this distinctive subtype. Identification of "driver" mutations is essential to understand the molecular mechanisms of HNSCC or both subtypes [4,18]. Therefore, based on the patient's specific genomic state, the treatments resulting from this design could target their tumors specifically and directly at the same time possibly eliminating unnecessary treatments, target prevention, decrease morbidity, and reduce treatment costs.

Chapter III

Materials and Methods

(This section was taken from the WRNMMC protocol IRBNet # 388953 entitled "Expression of p16, p53, EGFR, and mTOR on HPV and non-HPV Associated Head and Neck Squamous Cell Carcinoma.")

Twenty two specimens of squamous cell carcinoma of the head and neck, with the exception of skin, that were diagnosed between January 1, 2001 and March 31, 2013 were identified through a search of electronic medical records (Co-PATH/CHCS/AHLTA) from Walter Reed National Military Medical Center (WRNMMC) and of the database of the Naval Postgraduate Dental School (NPDS), Oral and Maxillofacial Pathology Department. Initial identification of cases entailed a natural language Co-PATH search for: "squamous cell carcinoma", "oral", "tongue", " floor of the mouth", "buccal", "pharynx", "oropharynx", "nasopharynx", "tonsil", "larynx" to identify all HNSCC diagnosed by the NPDS Oral and Maxillofacial Pathology Department or WRNMMC Anatomic Pathology Department. For each case selected, 10 unstained slides were requested for immunohistochemistry and *in situ* hybridization.

Clinical data were extracted into a database (Appendix A). This database was de-identified (the accession number, name, SSN, and DOB was removed) by assigning a unique study ID number to each case. Absent or inadequate biopsy specimens or medical records were excluded from the study. Associate investigators transferred the slides to the National Institute of Dental and Craniofacial Research (National Institute of Dental and Craniofacial Research) to perform immunohistochemistry and *in situ* hybridization to quantify the expression of pAKT437, pS6, p53, EGFR, p16 and HPV. The presence of HPV was determined using *in-situ* hybridization. HNSCC and HPV-SCC were compared for expression of p16, p53, pAKT437, pS6, as well as age at diagnosis, location, gender, race, smoking and alcohol consumption history, and clinical outcome information obtained from AHLTA.

The slides were subjected to immunohistochemistry to quantify the expression of pAKT437, pS6, p53, EGFR, and p16 and *in situ* hybridization as follows:

> Paraffin sections were be incubated at 65°C for 15-30 minutes, dewaxed in 3 charges of SafeClear II (Fisher), 5 minutes each, and hydrated with graded alcohols (100, 95, 70), 2 changes, 5 minutes each. Endogenous peroxidase was blocked by incubating for 20 minutes in 3% H_20_2 in 70°C ethanol. The antigens were then be retrieved in 10 mM citric acid (2.1 gr/L) and heated in a microwave, 2 minutes at 100% of the microwave's power for 18 minutes. The slides were allowed to cool for 15 minutes and washed extensively with distilled water, followed by 3 charges of phosphate-buffering saline (PBS), 5 minutes each. After blocking with 2.5% bovine serum albumin (BSA) in PBS (room temperature, RT) for 30 minutes, the slides were incubated with the first antibody overnight at 4° C, washed with PBS, 3 changes, 5 minutes each, and incubated with the secondary or link antibody, 1:400, for 30 minutes at room temperature (RT).

Following several washes with PBS, the slides were incubated with avidn-biotin complex (ABC) peroxidase solution (Vector Lab, CA), using Vector's ABC method in 2.5% BSA in PBS for 30 minutes at RT. The slides were then extensively washed with PBS and the reaction developed with 3,3-Diaminobenzidine (DAB) under microscopic control. The reaction was stopped with distilled water. Mayer's hematoxylin was used to counterstain, and the slides was washed 15 minutes in running tap water to bluish. The slides were then dehydrated in graded alcohols (70, 95, 100), 2 changes, 5 minutes each, then cleared in Safe Clear II, 2 changes, 5 minutes each, and mounted in permanent mounting media.

In situ hydridization was used for HPV detection. Formalin-fixed, paraffin-embedded samples were incubated with the probe cocktail containing HPV family genotypes (usually HPV genotypes: 16, 18, 31, 33, 35, 39, 51, 52, 56, 58, and 66). Visualization was evaluated using the manufacturer's detection systems. The system used indirect detection of antigen, beginning with a rabbit antidinitrophenol primary antibody, then by a secondary biotinylated antibody and followed lastly by streptavidin-conjugated alkaline phosphatase as the chromogenic enzyme.

All slides were scanned at 40x using an Aperio CS Scanscope (Aperio, Vista, CA) and quantified. Immunoexpression of p53 was quantified as positive or negative, while EGFR, p16, pAKT473, and pS6 were quantified according to percent of cells stained. *In situ* hybridization of HPV 16/18 was characterized as positive or negative. Fields for each stain were added to the previously created de-identified database (Appendix A).

Chapter IV

RESULTS

The results for this interim analysis, extrapolated from Table 1, showed that 85% of patients were male and a large percentage Caucasian. The mean age was 52 years. Follow-up information for patient outcome was available for time spans between 2-10 years. Half of the tumors were located in the oropharynx and half in the oral cavity. A small number of patients, 2/22, succumbed to their disease. There was a significant proportion of patients (55%) that were at a clinical or pathologic stage IV at the time of their diagnosis and 85% of those were p16 (HPV) positive.

By immunohistochemistry, p16 the surrogate marker for HPV, 13/22 (almost 60%) were positive and 9/22 (about 40%) were negative. 8 of 22 cases (36%) were reactive to the p53 nuclear immunostain and 14/22 (63%) were negative. The pS6 marker, which represents the over-activity of the mTOR pathway, presented as a percentage of positivity, did not show a significant variability in this early cohort.

Subject Demographics

Study ID #	G	Age	Ethn	Mil	TH	AH	Idx	1	Stage	Tx	F/u data	Outcome	p16	p53	EGF	pS6	pAKT
1	M	21			4py	n/a	2006	LT	n/a			n/a	1	0		60%	
2	M	46					2007	LT	Iva	SR	8YRS		0	0		50%	
3	M	55					2012	T	Iva	SRC	3YRS	DWD	1	1		50%	
4	M	51	ALEU										1	1		40%	
5	M	51	B	ADUSN	CHEW	3D/D	2014	T	I	S	1YR	AW/OD	1	0		80%	
6	F	48	W	RUSN	1.5PY	0	2007	VT	III	S	8YRS	AW/OD	0	1	Low	40%	
7	M	52	n/a	n/a	n/a	n/a	2007	DT	n/a		8YRS	n/a	1	1*		30%	
8	M	57	W	RUSA	90PY		2005	BOT	Ivb	RC	10YRS	DWD	0	0		50%	
9	M	58	W	USMC	0	4/WK	2011	BOT	IVa	RC	4YRS	AW/OD	1	1*		25%	
10	M	52	W	USMC	17PY	Occ.	2008	Oro	IVa	RC	7YRS	AW/OD	1	1		20%	80%
11	M	52	W	USN	0	0	2012	BOT	IVa	RC	3YRS	AW/OD	1	1*		35%	
13	M	48	W	USN	0	0	2008	BOT	IVa	SRC	7YRS	AW/OD	1	1*		15%	70%
15	M	57	n/a	P	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0	0		60%	
16	M	38	n/a	P	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0	0		70%	
17	M	50	W	USA			2012	BOT	IVa	RC	3YRS	AW/OD	1	1*		45%	
18	M	46	W	USA	0	0	2013		IVa	SR	2YRS	AW/OD	1	0		30%	
19	M	54	W	USA	0	0	2013	PT	IVa	SRC	2YRS	AW/OD	0	1		60%	
20	M	56	W	USAF	0	0	2013	BOT	IVb	RC	2YRS	AW/OD	1	1^		40%	
21	F	67	W	CIV	0	0	2012	LT	I	S	3YRS	AW/OD	0	1		60%	
22	F	60	B	CIV	0	0	2013	AT	I	SR	2YRS	AW/OD	0	1		40%	
23	M			USA	DIP	Y		T					1	0		70%	
24	M	56	B	USMC	0	Y	2007	TON	IVa	SRC		AW/OD	0	1		50%	
		52															
		52															
		51.19															

AwD=alive with disease; Aw/oD=alive without disease; DwD=deceased with disease; DOC=deceased of other causes; DU=data unavailable; VT=ventral tongue; LT=lateral tongue; FOM=floor of mouth; S=surgery; R=radiation; C=chemotherapy 1=positive 0=negative

Table 1. Raw Data Sheet

Chapter V

Discussion and Conclusion

A high percentage of HPV positive oropharyngeal squamous cell carcinomas were found in this study. Although this was a preliminary finding it was significant when compared to the largest study of HPV-SCC to date wherein a population of 147 patients demonstrated 18% positivity to HPV testing [13]. The findings of this study may indicate a potential difference experienced in our military when compared to the general population, specifically, that it is at higher risk for HPV-SCC, possibly due to an increase in sexual partners and/or behaviors.

Hypothetically, this evidence may support a need for a program of regular HPV vaccination-regimens for sexually active military personnel and their dependents. Additionally, since our findings of a predominantly male population is in line with the literature, preliminary consideration of prophylactic tonsillectomies on males who test positive for specific HPV subtypes may be worthwhile. The reactivity of pS6, though not significant between the HPV+/- cohorts, still demonstrates over activation of the mTOR pathway in neoplastic cells and may warrant the use of the inhibitor rapamycin or analogs for treatment and management of pS6+ SCCs. Rapamycin remains the most effective treatment for mTOR inhibition. The high percentage of Stage IV lesions at diagnosis, supports the notion that these lesions are difficult to detect early in their pathogenesis due to their location deep within the tonsillar crypts. New methods of early detection, which have yet to be developed, could increase both prognosis and outcome. Finally, a population seems to be emerging which is rarely mentioned in the literature, of patients whose tumor cells react positively to both p16 and p53. This group is of great interest; however, there is currently no existing research to explain this pattern. So what could this possibly suggest? Does the pathogenesis in the tumors of these patients work synergistically or does it demonstrate that patients who have a higher number of sexual partners and who smoke cigarettes have a different prognosis than those who are just HPV positive? Early findings are demonstrating that the individual neoplastic cells appear separately positive for p16 and p53.

In conclusion, efforts to collect specimens and data, along with the ongoing immunohistochemical staining, will continue until 120 cases have been reached. The entire cohort will then be evaluated in its entirety by comparing the staining results with corresponding demographic data. For this analysis, the EGFR and pAKT (the second stain that represented the mTOR pathway) failed due to the antibody not binding to their antigenic binding sites. This

problem has been attributed to the use older antibodies. New antibodies have been ordered and the immunohistochemistry will be repeated on additional unstained material prepared for just such an occasion.

Appendix A. Data Collection Sheet

DEMOGRAPHICS

Last name:______________________First Name:__________________
DOB:___/___/___ Designator and last 4 of SSN:__________________________
Biopsy Accession Number: __________________________

- -Once Data Collected Cut Here - - - - - - - - - - - - - - -

Study Number: _____________ Accession Number:_____________ Gender:
M___ F____ Age at diagnosis ______
Ethnicity: White___ Black ____ Asian ___ Other _____
Military Status: ______

SOCIAL HISTORY

Tobacco History (pk/yrs): ________
Alcohol History: _____________________

CLINICAL DIAGNOSIS

Date of initial Diagnosis: ______________
Site of primary SCC: ______________________
Stage: _______________
Treatment: Surgery___ Radiation___ Chemo ____
Length of follow-up data available _______
Outcome: Alive without disease _____ Alive with disease_____
Deceased with disease_____ Deceased from other causes______
Data unavailable______

LABORATORY DATA

HPV: ___________

p16: ____________

p53: ____________

EGFR:___________

pS6:____________

pAKT437:________

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